EXAMPLE #4

DETECTION OF DNA SEQUENCES BY DIRECT COMPETITION

A first oligomer of **40** bases containing a terminal amine was covalently coupled to a silanized glass coverslip as described for anti-BSA in Example 1A, A second oligomer also containing a terminal amine and having a sequence complementary to that of the first oligomer was coupled to latex beads as in Example 2 at a concentration previously determined to permit the formation of only one hybrid molecule of the complementary oligomers. The force necessary to separate this hybrid was experimentally determined. Detection of a competing oligomer was accomplished by adding to the system a solution of a single stranded oligomer having a sequence complementary to the sequence of either the oligomer immobilized on the coverslip or fire oligomer on the latex bead at a concentration of 1–1000 molecule/microliter. This added oligomer hybridized with its complementary oligomer immobilized on the coverslip or the head, thereby blocking the binding of the competing oligomer immobilized on the latex bead or coverslip, respectively.

EXAMPLE #6

DETECTION OF LARGE PIECES OF DNA

using two single-stranded DNA oligomers having different, noncomplementary sequences, with one oligomer attached to the glass slide and the other oligomer attached to the bead, we were able to detect a unique sequence in a large piece of single-stranded DNA in solution containing both sequences in different locations on the strand. The strand was able to bind to the oligomer on the coverslip and to the oligomer on the bead, thus being caught between the two. The force required to break these bonds exceeded the background, Quantifying the amount of single-stranded DNA in solution can be accomplished with a competitive binding assay using a DNA oligomer having a sequence complementary to the sequence of either the oligomer on the coverslip or the oligomer on the bead to compete with the binding of the DNA strand to the oligomer immobilized on the bead or coverslip, respectively. Detection of 1–100 DNA copies can be performed in this fashion. Therefore, as readily apparent to those skilled in the art, the apparatus of the present invention can be used to detect and quantitate nucleic acid molecules having a known sequence.

The above examples are intended to be illustrative rather than limiting. Those skilled in the art who have reviewed this specification will readily appreciate that the techniques described herein can be adapted to detection and quantitation of other analytes without departing from the scope of the present invention. It will be readily apparent to those skilled in the art that by use of these techniques, it is possible to detect and quantitate any and all nucleic acid sequences, antigens, antibodies and other analytes at extremely low concentrations, i.e. as low as about 1 molecule of analyte per microliter of sample. Detection of analytes present at concentrations less than 1 molecule per microliter can be accomplished with the apparatus of the present invention by allowing sufficient time for the specific binding reaction to occur or by including means of moving the ligand into contact with its corresponding ligand binder. It will also be readily apparent that the ligand and the ligand binder can be on separate beads and that ligands or their corresponding binding agents can be arranged on a "chip" to permit multiple analysis for the desired analyte, e.g, antigen, antibody, DNA target, etc. Thus using mixed beads with different antigens attached or beads of different color, etc., it is possible to run several assays on one sample. This technology provides at rapid, inexpensive, automatable, method for detecting individual molecules of interest to the analytical and clinical chemist. It is applicable for detecting low concentrations of infectious organisms, such as HIV and other viruses, oncogenes, growth factors and other materials where sensitivity is a problem. It can be a major competitor of polymerase chain reaction (PCR), ligase chain reaction (LCR), Q-beta replicase and similar technologies for assays of very low concentrations of nucleic acid material.

We claim:

1. A method of detecting and quantitating an analyte in a sample, the method comprising:

(a) providing a laser light source for emitting a beam of laser light;

(b) providing first and second bodies, one of the first and second bodies being adapted to be manipulated by the beam of laser light and the other of the first and second bodies being immovable by the beam of laser light;

(c) adhering to the first body a first reagent selected from the group consisting of a substance identical to the analyte and analyte competitors;

(d) adhering to the second body a second reagent capable of binding to both the first reagent and to the analyte, but to only one at a time;

(e) bringing the first and second bodies into sufficient proximity in the presence of the sample to cause a competition between the first reagent and the analyte in the sample for binding to the second reagent, the competition resulting in a stable complex between the first reagent and the second reagent, the complex holding the first and second bodies together;

(f) breaking the complex between the first reagent and the second reagent by use of the beam of laser light to separate the first and second bodies;

(g) determining a force necessary to carry out step (f); and

(h) determining a quantity of the analyte in the sample from the force determined in step (f).

2. A method as in claim **1**, wherein the first body is a bead between about 0.5μ to about 100μ in diameter and comprises a material that does not adsorb light at the wavelength of the beam of laser light.

3. A method as in claim **2**, wherein the second body is selected from the group consisting of a glass coverslip and glass slide.

4. A method as in claim **3**, wherein the second body is coated with a silane coupling agent for forming a covalent bond with the second reagent.

5. A method as in claim **1**, wherein:

the analyte is an antigen; and

the second reagent is an antibody capable of binding specifically to the antigen.

6. A method as in claim **1**, wherein the analyte and the second reagent comprise nucleic acids having sequences that are complementary to each other.

7. A method as in claim **1**, wherein the step of adhering the first reagent to the first body comprises:

(i) adhering a third reagent to the first body, the third reagent being reactive with the first reagent; and

(ii) exposing the first body containing the adhered third reagent to the first reagent to cause the adhered third reagent to react with the first reagent.

8. A method as in claim **7**, wherein the third reagent comprises a carboxyl group.